A New Addition to the Folding Repertory: An Engineered Five-Stranded Tryptophan Zipper

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Coiled-coil motifs are ubiquitous mediators of specific protein-protein interactions through the formation of interlocking hydrophobic seams between α-helical chains. We engineered a "Trp-zipper" protein with tryptophan (Trp) residues at 14 first and fourth heptad positions. The protein forms a discrete, stable, α-helical pentamer in water at physiological pH. Its 1.45 Å crystal structure reveals a parallel, five-stranded coiled coil, a new type of "knobs-into-holes" packing interaction, and an unusual ~8 Å diameter axial channel lined with indole rings. The engineered Trp-zipper pentamer expands current views of coiled-coil assembly, molecular recognition, and protein engineering, and may serve as a soluble model for membrane ion channels.

Coiled coils are one of the most common motifs in protein-protein interactions and have been used as the basis for the design of helical bundles and many related structures. They consist of entwined alpha helices that form a hydrophobic seam based on nonpolar side chains located at the first (**a**) and fourth (**d**) positions in a characteristic seven-amino-acid sequence repeat, called a heptad. Despite their simple sequence pattern, coiled coils exhibit great diversity in the number and orientation of the chains involved. Proteins composed of two to twelve "strands" are known, and general principles for assembling dimers as well as higher-order structures, including hexamers and dodecamers, have been deduced.

About five years ago, we became interested in the *E. coli* lipoprotein (a protein-lipid molecule, denoted Lpp) that contains a three-stranded coiled-coil domain (Lpp-56) embedded between the outer cell membrane and the periplasmic peptidoglycan. Surprisingly, the crystal structure of this trimeric domain shows that the superhelical structure is not a uniform cylinder, but has a pinched-in region where three adjacent alanines (small hydrophobic amino acids) occupy the normally bulky nonpolar side chains at the **a** and **d** heptad positions. This unusual flexibility in the local helix geometry has not been seen in GCN4 leucine-zipper models, and led us to undertake a systematic analysis of the effect that the number and size of the side chains at the **a** and **d** positions might have on the structure and stability of the protein. Replacing *all* of the **a** and **d** residues with alanine, for example, completely unfolds the protein in solution, although the trimer is stable in crystals. This effect had not been reported before, to our knowledge: Intermolecular interactions in the crystal were sufficient to stabilize a coiled-coil trimer that could *not* fold in solution. Even when we cross-linked the chains covalently, the protein in solution remained monomeric. The monomer provided a useful model for defining the folding pathway in this system because we could identify a nascent helical sequence near the carboxyl terminus that unfolded as the temperature increased.

To explore the effects of helix geometry on coiled-coil formation, we engineered a Lpp-56 variant that contains exclusively Trp residues at the **a** and **d** positions (**Figure 1**). This Trp-zipper protein (denoted Trp-14) forms a stable pentameric coiled coil in solution. Its 1.45 Å crystal structure reveals a never-

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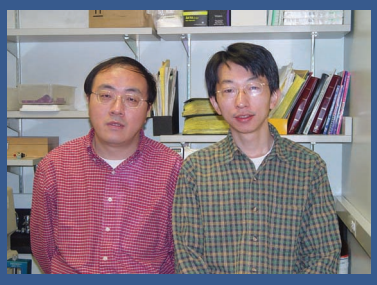

Authors (from left) Min Lu and Jie Liu

seen-before interface between five parallel helices formed by interacting Trp residues. Only the residues at the **a** positions obey classical "knobs-into-holes" packing, while the **d** positions exhibit a more perpendicular stacking arrangement of the indole rings. This result suggests that the **a** and **d** layers in larger diameter helical barrels may unequally contribute to the stability of the structure. The Trp-14 structure also contains an irregular hydrophobic cavity (with a diameter of 8 Å) running along the long axis. This engineered Trp-zipper structure shows that we have not achieved a complete understanding of the principles of coiled-coil assembly. We are exploring additional variants of Lpp-56 that will help us to do so. Meanwhile, we note that the channel in Trp-14 may serve as a soluble model for membrane ion channels, and may help determine the thermodynamic effect of binding various ligands in such a channel.

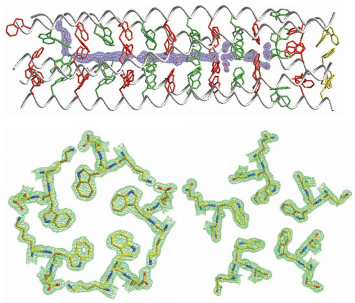

Figure 1. Crystal structure of Trp-14. The top panel shows a side view of the pentamer. The bottom panel shows the cross-section of the pentamer in the W18 (**a**, left**)** and W21 (**d**, right**)** layers. The view is from the amino terminus of the Trp-14 pentamer.